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Roles play by Toll-like receptors 2 and 4 in acute *Pseudomonas aeruginosa* lung infections in mice

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Aims: Toll-like receptors (TLRs) have been implicated in defense against microbial infections. Indeed, animal models have demonstrated that susceptibility to a number of gram negative pathogens is linked to TLR4 and thus lipopolysaccharides (LPS) of many gram negative bacteria have been implicated as virulence factors in a number of diseases. The aim was to assess the role of this pathogen-associated molecular pattern as it is exposed on intact *Pseudomonas aeruginosa*,
Methods. The susceptibility of mice lacking TLR4 or both TLR2 and 4 was examined in a model of acute Pseudomonas pneumonia.
Results: The mutant mice were not hypersusceptible to the Pseudomonas challenge and mounted an effective inflammatory response that cleared the organism, despite low levels of TNF-alpha and KC in the airways. Microorganism and neutrophil counts were similar in control and deficient TLR mice at 6 and 24 hours after infection. MyD88-/- mice were however hypersusceptible with 100% of mice dying within 48 hours with a lower dose of *P. aeruginosa*. Of note there was normal levels of interleukin-6 (IL-6) and granulocyte colony-stimulating factor (G-CSF) in the airways of the TLR mutant mice that was absent from the MyD88 deficient mice.
Conclusion: The susceptibility of mice to *P. aeruginosa* acute lung infection does not go through TLR2 or TLR4, negating Pseudomonas LPS as an important virulence factor in acute lung disease caused by this organism. Furthermore the resistance to infection in these mice may be linked to IL-6 and G-CSF production.

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Expression of Toll Receptors 2 and 4 in response to Burkholderia cepacia complex (BCC) infection of epithelial and macrophage cells

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Activation of Toll-like receptors (TLRs) on airway epithelial cells has been shown to be involved in the regulation of a variety of genes including those encoding cytokines. Toll receptors constitute the first line of defence against many pathogens. The aims of this study were to analyse TLR 2 & TLR4 expression on epithelial and macrophage cells in response to B.cenocepacia BC7 & B.multivorans LMG10310 infection. A549 epithelial cells and U937 macrophage-like cells were seeded into 6-well plates prior to infection with either BCC strain or 10 ng/ml LPS for 4 & 24 hrs. Cell surface and intracellular TLR2 & TLR4 expression were analysed by flow cytometry. The results demonstrate that A549 epithelial cells constitutively express low levels of surface TLR 2 & 4 and do not increase in response to BCC strains or LPS. In contrast, intracellular levels of TLR4 were 10 fold higher than surface TLR4 levels. Cells infected with B.cenocepacia BC7 significantly down regulated intracellular TLR4 expression ($P<0.026$) after 24 hrs infection. Infection with B.multivorans LMG10310 or LPS also induces a down regulation of intracellular TLR4 expression, but this is not significant. U937 cells also show a down regulation of TLR2 and TLR4 expression after BCC or LPS stimulation. BCC strains are known to invade A549 epithelial cells and induce a proinflammatory cytokine response suggesting that upon infection of the cells, signalling through intracellular TLR4 may be a contributing factor in the proinflammatory response that ensues. Future work will involve examining mRNA expression of TLR2 & 4 and intracellular signalling cascades involved in epithelial and macrophage responses to BCC infection.

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Glutathione modulation of MMP-9 and TIMP-1 production in respiratory epithelial cells

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Elevated levels of MMP-9 have been identified in Cystic Fibrosis (CF). Respiratory epithelium constitutively expresses MMP-9, which can be upregulated by inflammatory stimuli such as TNF-alpha. MMP-9 is inhibited by TIMP-1. Glutathione (GSH) is a major intracellular antioxidant that can modulate the inflammatory response. We hypothesized that augmentation of intracellular glutathione by GSH supplementation would diminish MMP-9 production.
Aim: To quantify respiratory epithelial cell MMP-9 and TIMP-1 production, basally and in response to stimulation with TNF-alpha (10 ng/mL), with and without GSH augmentation.
Methods: Airway epithelial cell lines expressing either wildtype (9HTEo-) or deltaF508 (CFTE29o-) CFTR.
Zymography: MMP-9 and Timp-1 activity were quantified by zymography and reverse zymography, respectively.
Results: There was a significant increase in MMP-9 production post TNF-alpha stimulation in both cell lines, with no significant change in TIMP-1 levels. Supplementation with GSH did not significantly change basal MMP-9 or TIMP-1 production. However stimulation with TNF-alpha in the presence of GSH supplementation demonstrated a significant reduction in MMP-9 production ($P<0.05$) with a significant increase in TIMP-1 production ($P<0.05$) in the CF cells. A similar, but non-significant, trend was identified in the wildtype cell line.
Conclusion: Glutathione augmentation resulted in both a decrease in MMP-9 production and an increase in TIMP-1 production by respiratory epithelial cells in response to stimulation by TNF-alpha. These changes could limit the parenchymal damage in CF. This is one other mechanism whereby therapies aimed at increasing intracellular GSH may be beneficial in CF.

Supported by Canadian CF Foundation; Cells: D. Gruenert

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Increased *Pseudomonas aeruginosa*-induced inflammatory response in epithelial cells expressing mutated CFTR

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The mechanisms by which defective CFTR protein produces airway disease are not fully elucidated. However, a growing consensus indicates that the CF airways are marked by an aberrant and dysregulated inflammatory response. In the present study the possibility that mutated CFTR protein may itself amplify the inflammatory responses to bacterial pathogens has been evaluated in C127 (mouse epithelial cells - CFTR w/t or ΔF508) and IB3 (human bronchial cells - CFTR ΔF508/W1282X,) cell lines. C127 cells were infected with a laboratory strain of *Pseudomonas aeruginosa* (PAO1) (0.05-5 cfu/cell) and ICAM-1 mRNA was quantitated 4 hours after infection by real-time PCR. PAO1 infection increases ICAM-1 transcription in a dose dependent manner. ICAM-1 mRNA induction is significantly higher in C127 CFTR ΔF508 than in CFTR w/t cells ($P<0.04$). A sharply increased response of C127 ΔF508 cells appears within 2 hours post infection, and this does not correlate with augmented bacterial binding to the cells, as tested with metabolically labelled [^{35}S]PAO1. IB3 and S9 cells (IB3 line corrected with CFTR w/t) were infected with PAO1 as indicated and mRNA levels of ICAM-1 and IL-8 were determined. Infection with PAO1 up-regulates both ICAM-1 and IL-8 transcripts. Induction of ICAM-1 mRNA, IL-8 mRNA and IL-8 protein are significantly higher in IB3 than in S9 cells ($P<0.03$, $P<0.007$, $P<0.008$). These results indicate that the expression of mutated CFTR is associated with augmented inflammatory responses upon infection by PA.

Supported by the Italian Cystic Fibrosis Research Foundation (FFC#14/2004) and Legge 548/93, Finanziamento Ricerca Fibrosi Cistica 2004.